

yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. For every 10 ml of yolk, 100ml of Tris buffer saline was added. The precipitate formed was removed by centrifugation. To the supernatant the precipitating solution of magnesium chloride and phosphotungstic acid was added and centrifuged again. The pellet discarded and to the supernatant now called the water soluble protein fraction 12% polyethylene glycol was added and incubated for 10 minutes and then centrifuged. The antibody precipitates out. 10 ml of 10mM phosphate buffer is added and the precipitate dissolved. The antibody solution is then cooled to 0°C and 10 ml of pre cooled ethanol added. The solution is centrifuged at 4°C and the sediment is dissolved in 10-mM phosphate buffer and dialyzed in phosphate buffer for 24 h at 4°C. Yield - 60%.

#### **EXAMPLE –2**

The lipid from the egg yolk was precipitated out twice using the precipitating solution of phosphotungstic acid and magnesium chloride from the supernatant and centrifuged.

Yield – 75%.

#### **EXAMPLE –3**

The pH of the water soluble protein fraction obtained after the removal of the lipids was checked and adjusted to pH 5.0 in order to further precipitate out the antibodies.

Yield – 80 –90%.

#### **EXAMPLE – 4**

The egg yolk was carefully removed from the eggshell without rupturing the yolk membrane. The entire albumin adhering to the membrane was washed off. And the egg yolk membrane was ruptured and transferred into a measuring cylinder through a funnel. For every 10 ml of yolk, 10 ml of distilled water was added. 0.15 % of kappa-carragenanin was added and left to stir for 30 minutes at room temperature. The solution was then filtered and centrifuged at 10, 000g for 15 minutes. The pH of the supernatant solution was set to pH 8.0 and then passed through the DEAE – sephacel column prepared with 20 mM phosphate buffer pH 8.0. The antibody was eluted with 0.2 M phosphate

buffer pH 8.0. The eluate was collected and the absorbency read at 280 nm. The peak fractions containing the antibody were pooled and stored at 4°C. Yield – 73 %.

The efficacy of these antibodies has also been inferred through this invention.

## **ADVANTAGES**

The main advantages of the present invention are:

- (a) The use of antibodies from the egg yolks of hyperimmunized hens (HEY antibody) for immunological procedures overcome the limitations associated with the polyclonal and monoclonal antibodies, because the present method provides a continuous supply of large quantities of consistent, high titer specific and sensitive antibody which can be easily collected and stored.
- (b) The sensitivity of the assay is equal or better than rabbit antibodies.
- (c) The method of immunizing the poultry birds is non-invasive and has a good affinity to the analyte.

The advantages of antibodies produced by chicken over rabbit are further illustrated in the tabular form as provided in the following Table I.

**TABLE I**

<b>PARAMETERS</b>	<b>LAYING HENS(4 NOS)</b>	<b>RABBITS(2-4 NOS)</b>
<b>A. MAINTENANCE COST IN Rs.</b>		
(a) Investment for cage	15,000	20,000
(ii) Price for each animal	60	75
<b>B. ANTIBODY PRODUCTION</b>		
(i) Monthly	40 mg/ml	7mg/ml
(ii)Amount of specific antibody	2000-2800mg upto 10%	200 mg 5%
<b>C. INFRASTRUCTURE</b> Area needed	Poultry birds	Small animals
<b>D. CHARACTERISTICS</b>		
i) Antibody sampling	Non-invasive	Invasive
ii) Purification	Affinity	Affinity
iii) Interference with rheumatoid factor	No	Yes
iv) Activation of mammalian complement	No	Yes